Vasoactive intestinal peptide/pituitary adenylate cyclase activating polypeptide, and their receptors and cancer

Terry W. Moody[a], *Bernardo Nuche-Berenguer*[b], *and Robert T. Jensen*[b]

Purpose of review
To summarize the roles of vasoactive intestinal peptide (VIP)/pituitary adenylate cyclase activating polypeptide (PACAP) and their receptors (VPAC1, VPAC2, PAC1) in human tumors as well as their role in potential novel treatments.

Recent findings
Considerable progress has been made in understanding of the effects of VIP/PACAP on growth of various tumors as well as in the signaling cascades involved, especially in the role of transactivation of the epidermal growth factor family. The overexpression of VPAC1/2 and PAC1 on a number of common neoplasms (breast, lung, prostate, central nervous system and neuroblastoma) is receiving increased attention both as a means of tumor imaging the location and extent of these tumors, as well as for targeted directed treatment, by coupling cytotoxic agents to VIP/PACAP analogues.

Summary
VIP/PACAP has prominent growth effects on a number of common neoplasms, which frequently overexpressed the three subtypes of their receptors. The increased understanding of their signaling cascades, effect on tumor growth/differentiation and the use of the overexpression of these receptors for localization/targeted cytotoxic delivery are all suggesting possible novel tumor treatments.

Keywords
cancer, glial tumors, lung cancer, neuroblastoma, PACAP, VIP

INTRODUCTION

Vasoactive-intestinal peptide (VIP), a 28-amino acid peptide [1], has sequence homology (67%) with pituitary-adenylate-cyclase-activating polypeptide-27 [2,3▪▪]. Their biologic actions are mediated by three classes of G-protein-coupled receptors (GPCR) (VPAC1, VPAC2 and PAC1) which are members of the class II/class-B secretin-like receptors [3▪▪,4,5]. Other family members include glucose-dependent insulinotrophic peptide, glucagon, glucagon-like peptide-1, glucagon-like peptide-2, peptide-histidine-methionine/valine/isoleucinamide, growth-hormone-releasing factor and secretin [3▪▪,4,5]. VPAC1, VPAC2 and PAC1 activations stimulate diverse signaling cascades, which principally are mediated by adenylate-cyclase, stimulation of PKA/EPAC proteins, as well as by activation of phospholipase-C-causing protein-kinase C stimulation and cellular-calcium changes (especially PAC1-activation) in many tissues [3▪▪,4,5]. Numerous PAC1-receptor splice-variants occur (both NH2-terminal and intracellular-loop-3 variants) and these can differ in their signaling cascades [3▪▪,5–8]. At present, the differential importance of the different PAC1-splice variants in normal physiology/pathophysiological conditions remains unclear [6,9].

Recently, a number of reviews have covered various aspects of their possible therapeutic use in diseases, as well as their roles in various physiological/pathophysiological conditions. These include their roles in inflammatory disorders [10–14]; immune/autoimmune diseases [10,11]; neurodevelopment/

[a]Department of Health and Human Services, National Cancer Institute, Center for Cancer Research, Office of the Director and [b]National Institutes of Health, National Institute of Diabetes, Digestive and Kidney Disease, Digestive Diseases Branch, Bethesda, Maryland, USA

Correspondence to Robert T. Jensen, National Institutes of Health, Building 10, Rm 9C-103, Bethesda, MD 20892, USA. Tel: +1 301 496 4201; fax: +1 301 496 0200; e-mail: robertj@bdg10.niddk.nih.gov

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KEY POINTS

- VIP and PACAP have prominent effects on growth (stimulatory or inhibitory) of a number of normal and neoplastic tissues and can function in an autocrine manner to affect growth.
- Many common neoplasms overexpress receptors for one or more of the three receptors mediating the actions of VIP/PACAP (VPAC1, VPAC2 and PAC1).
- There is increasing interest in using tumor overexpression of VIP/PACAP receptors for targeting tumors both for imaging and for receptor-mediated cytotoxicity (using nanoparticles, coupled to radiolabeled or cytotoxic compounds).
- This latter approach is receiving particular attention with cancers of the breast, lung, prostate, CNS, colon and with neuroblastomas.

behavior [15–17]; neuroprotection/neurodegenerative disorders [18–23]; pulmonary diseases (asthma and so on) [24]; sepsis [25]; learning/memory disorders [26]; central nervous system (CNS)/neurological disorders [14,21,27–29]; diabetes [30,31]; migraine [32,33]; stress-responses/disorders [34,35]; renal injuries [20] and general reviews of their signaling/pharmacology as well their roles in physiology/pathophysiology [3▪▪,5,6,36,37]. In this review, the roles of VIP/pituitary adenylate cyclase activating polypeptide (PACAP) in neoplastic processes, which has generally not been dealt with recently [38,39], will be concentrated on. Particular attention will be paid to VIP, which has received the most attention, both in its effects and signaling in neoplastic growth, as well as in its roles in tumor-imaging and possible therapeutic roles in neoplastic processes.

GENERAL: VIP-PACAP STRUCTURE, RECEPTORS AND PHARMACOLOGY

PACAP occurs in two biologically active forms, PACAP-27 and a COOH terminally extended form, PACAP-38 [3▪▪,6]. PACAP-27/38 are processed from a 176-amino acid precursor (preproPACAP) with the PACAP gene located on chromosome 18p11, consisting of 5-exons/4-introns [3▪▪,6]. VIP is a 28-amino-peptide and is processed from a 170-amino acid precursor (preproVIP) which also yields peptide-histidine-methionine/valine, and is encoded by a gene located at chromosome 1p11, with 7-introns/6-exons [3▪▪,5].

VIP has high-affinity for VPAC1/VPAC2 receptors, whereas PACAP-27/38 are nonselective, having high affinity for all three receptor subtypes [3▪▪,4,5,40▪▪,41]. The investigation of the role of these receptor subtypes in physiological/pathophysiological conditions has been hampered because of difficulty in developing selective ligands for each receptor subtype. There are no potent nonpeptide antagonists for these receptors [3▪▪,4,5,40▪▪,41]. In addition, whereas selective peptide agonist/antagonists exist for VPAC receptors, for the PAC1 receptor, no potent, selective antagonists exist [3▪▪,4,5,40▪▪,41]. Furthermore, until recently, the only selective, potent PAC1 agonist was maxadilan, a 61-amino acid peptide isolated from sand-flies [42], which is difficult to synthesize and therefore, rarely used [3▪▪,40▪▪]. Recently, a number of studies report PACAP analogues with differing selectivity for the PAC1 receptor, as well as enhanced stability [40▪▪,43,44]. A recent study [40▪▪] reporting the greatest selectivity, involved synthesizing conformationally restricted PACAP analogues. A number of PACAP analogues were identified [40▪▪] which were biologically active, potent and selective for PAC1. The most selective analogues were more than 75-fold PAC1-preferring over VPAC1 and more than 800-fold over VPAC2 [40▪▪]. At present, these new selective PAC1 agonists have not been investigated in physiological/pathophysiological conditions.

GENERAL: VIP-PACAP AND RECEPTORS IN TUMORS

VIP immunoreactivity (VIP-IR) occurs in a number of tumors [45▪] and VPAC1 is overexpressed, resulting in high densities, in numerous cancers including bladder, breast, colon, liver, lung, pancreatic, prostate, thyroid and uterus-cancer [39,45▪,46,47▪▪,48]. In contrast, VPAC2 has been less well studied, but is present in gastric leiomyomas; thyroid, gastric/pancreatic adenocarcinomas; lung tumors, various sarcomas and neuroendocrine-tumors [46,48–50]. PACAP immunoreactivity occurs in colon, lung and prostate cancers [45▪,51]. PAC1 is present in tumors of the brain, breast, colon, lung, neuroendocrine, pancreas, pituitary, prostate as well as neuroblastomas/pheochromocytomas [45▪,46]. These results suggest that VIP/PACAP may play important roles in the modulation of growth/differentiation of many human tumors [45▪,52]. VIP stimulates growth of many tumors including breast, lung, pancreas, prostate, in addition to various CNS tumors (gliomas, astrocytomas and so on), [38,45▪,53,54▪,55–57], as well as having an inhibitory effect on the growth of other tumors (retinoblastoma and renal cell) [58,59]. PAC1 activation also stimulates the growth of a number of tumors including neuroendocrine, brain, breast, prostate, pancreatic, colon and lung [45▪,60▪,61,62]; differentiation of pheochromocytoma cells [63] and has a

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growth inhibitory effect on some neoplasms (medulloblastoma and gliomas) [64,65].

IMAGING/TARGETED DELIVERY OF NEOPLASMAS USING OVEREXPRESSION OF VIP/VPAC RECEPTORS

Overexpression of somatostatin receptors (sst1–5) by neuroendocrine tumors/other tumors (CNS, etc.) is currently widely used for their imaging and when combined with PET and computed tomography (CT) is the most sensitive localization method [66]. Furthermore, when combined with sst-ligands conjugated to cytotoxic-agents such as ^{90}Yttrium and ^{177}Lutetium, sst overexpression can be used therapeutically in patients with advanced disease [66,67▪▪]. A similar approach is being investigated for a number of tumors (breast, prostate, etc.) overexpressing other GPCRs (bombesin, chemokine, gastrin, etc) [68–71] including VPAC-overexpressing tumors (especially breast and prostate) using various radiolabeled VIP analogues [39,47▪▪,72,73]. Recent studies report the successful tumor localization using various radiolabeled VIP analogues for experimental studies in animals for cancers of colon [74–76], prostate [77] and breast [78]. Furthermore, studies in humans report localization using various radiolabeled VIP analogues for breast cancer [78,79], pancreatic cancer [80,81], intestinal adenocarcinomas [73], neuroendocrine tumors [73,82] and colorectal cancer [83]. In one study [80], no imaging was seen with biological, active, radiolabeled VIP analogues in patients with pancreatic, colorectal adenocarcinomas or neuroendocrine tumors, and in-vitro autoradiography demonstrated that these tumors did not overexpress VPAC1 [80].

Furthermore, a number of studies have used the overexpression of VPAC/PAC1 receptors on tumors to deliver cytotoxic agents [84,85▪,86–89]. One novel approach coupled nanoparticles to VIP to target the VPAC-overexpression on various cancer cells [84,85▪,86–88]. The nanoparticles are filled with various cytotoxic agents, which cause cell-death in different cancers [85▪,86–88]. In the specific tumor sections below, results with a number of these studies will be discussed in more detail.

VIP/PACAP: LUNG CANCER

VIP receptors occur in 60% of lung carcinoma cells, with VIP-IR, pro-VIP forms and COOH terminal extended forms frequently found [39,45▪,46]. VPAC1/VPAC2 mRNA is found in 51 and 48% of lung cancer surgical specimens [48]. A number of recent basic science and animal studies provide insights into the signaling cascades and effects of VIP on lung cancer cells, which may yield therapeutic approaches.

Small cell lung cancer (SCLC) is a lung-cancer with endocrine-features, which kills approximately 25 000 U.S. citizens annually. SCLC is treated with chemotherapy/radiation therapy [90]. This therapy is initially effective, but relapses occur and the median survival time is less than 1 year. Addition of VIP to SCLC cells stimulates secretion of bombesin-like peptides in a cAMP-dependent manner [91]. Both VIP receptor and PACAP receptor antagonists inhibit the growth of SCLC cells [45▪,92–94]. A lipophilic, VIP analogue which functions as an antagonist (Stearyl-Nle(17)-neuroten-sin(6–11)VIP(7–28) [N-stearyl, Nle17)VIP hybrid] [93], inhibited lung cancer growth and had synergistic inhibitory-effects with chemotherapeutic agents. These results raise the possibility that this approach could be of clinical value [94].

Recent studies demonstrate that many G protein-coupled receptors stimulate tumor growth by transactivating the epidermal-growth factor receptor (EGFR) [95▪▪]. Recent studies on non-small-cell lung cancer cells (NSCLC cells) demonstrate that PACAP stimulates their growth by transactivating EGFR [55]. Transactivation requires phospholipase-C, and not adenylate-cyclase, but stimulation of matrix-melloproteinases, Src-kinases, transforming growth factor-α release and generation of oxygen free radicals [55]. With other GPCRs (bombesin and neurotensin) [95▪▪,96▪▪,97] inducing growth in lung cancer cells, the simultaneous inhibition of the GPCR by an antagonist and a tyrosine-kinase inhibitor (gefitinib, etc.) leads to potentiated growth inhibitory effects. These findings raise the possibility that a similar strategy could be considered with VIP/PACAP receptors on these tumor-cells.

After binding of VIP to VPAC1, the VIP–VPAC1 complex is internalized to endosomes [3▪▪,4,5]. This raises the possibility that the VIP receptor can be used to target cytotoxic agents to the tumor cell overexpressing the receptor, in a similar matter to that used in radioimaging studies. VIP ellipticine is composed of VIP coupled to ellipiticine(E), (a topoisomerase-II inhibitor) and activates VPACs in NCI-H1299 NSCLC cells [98] and an analogue, VIP-LALA-E, reduced cellular-viability [98]. It was proposed [98] that the VIP-LALA-E was metabolized in the lysosomes, releasing cytotoxic E into the nucleus of NSCLC cells, preventing unwinding of the DNA, impairing DNA replication. This is another example of how the overexpression of VPAC1 by lung cancer cells could be used as a molecular-target for cytotoxic agents [45▪].

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VIP/PACAP: BREAST CANCER

Breast cancer cells have VIP/PACAP receptors in up to 100% of cases [46] and have high densities of VPAC1 and its mRNA [39,45▪,99]. VPAC1, VPAC2 and PAC1 are reported in breast cancer tumors [39,45▪,100]. A number of results support a prominent role for VPAC/PAC1 activation in breast cancer growth. In various breast cancer cell lines, VIP and PACAP have been shown to activate adenylate cyclase, increase vascular endothelial growth factor (VEGF) expression and secretion, stimulate growth and the effects on the various cells inhibited by either VPAC1 antagonists or PACAP(6–38), a low affinity, but specific PAC1 inhibitor [39,45▪,52,94,101]. Furthermore, the addition of a VPAC inhibitor to various breast cancer cell lines potentiated the ability of taxol to inhibit proliferation [94]. VIP stimulates transactivation of both EGFR and the related receptor, HER2/Neu in breast cancer cell lines [102,103] and the addition of PAC1-siRNA inhibited the stimulatory effect of VIP on secretion of VEGF and the transactivation of EGFR and HER2/Neu.

The overexpression of VPAC receptors on breast cancer cells has recently been used both to image breast cancer cells and to target therapeutic agents to them. In nude mice studies with breast cancer xenografts (T47D human breast cancer cells), ^{18}F-^{15,21}Arg-VIP [104,105] and ^{64}Cu-TP3982 (VIP analogue with agonist activity) [78] were reported to show enhanced localization to the cancer cells. Subsequently, four synthetic analogues of VIP and PACAP (TP3939, TP3982, TP4200 and TP3805), which had high affinities for VPACs and PAC1-receptors (Kd-0.72–3.3 nM), were labeled with ^{64}Cu and which showed enhanced binding to human breast cancer tumor cells [106]. Current imaging modalities miss up to 30% of breast cancer and do not distinguish benign from malignant tumors [107]; therefore, more sensitive imaging methods are needed. In a study in MMTVneu mice, which spontaneous develop breast cancers, which resemble human breast cancers in that they also overexpressing VPAC-receptors, the efficacy of ^{64}Cu-VIP was compared with CT scanning and ^{18}F-PET scanning [107]. ^{64}Cu-TP3805 identified all malignant tumors that overexpressed VPAC, was more sensitive than the other imaging modalities and did not identify the benign tumors that did not overexpress VPA receptors [107]. This result led the authors to propose that PET scanning with this radiolabeled VIP analogue has potential for use in patients with early or metastatic breast cancer. Studies in humans ^{99M}Tc-labeled VIP [79] identified tumors in five patients with breast cancer and there was good concordance with results of CT and MRI localization.

To target breast cancer cells, camptothecin [(CPT), a topoisomerase I inhibitor)] was coupled to a VIP analog (Ala^{2,8,9,19,24,25,27}, Nle^{17}, Lys^{28})VIP, [(A-NL-K)VIP] and the resulting (A-NL-K)VIP-L2-CPT was cytotoxic for MCF-7 breast cancer cells [108]. In this study [108], it was proposed that the (A-NL-K)VIP-L2-CPT was metabolized in the lysosome and the CPT was released which inhibits the unwinding of DNA and its subsequent replication. Many of the drugs used to treat breast cancer have limited solubility, and to overcome this, long circulating, sterically stabilized phospholipid micelles (SSMs) have been developed which function as nano-size drug carriers [85▪]. SSMs containing paclitaxel or 17-allylamino-17-demethyoxy-geldanamycin (an inhibitor of heat shock protein 90) have been targeted to breast cancer cells by coupling to VIP and shown to be cytotoxic [86–88,109]. Breast cancer stem cells, which are important in tumor initiation, propagation, regeneration and resistance to conventional therapies [85▪], overexpress VPAC-receptors [85▪] and this has been used to target cytotoxic agents to them. In this study [85▪], a novel approach was used with SSMs containing curcumin (a natural polyphenol with antiproliferative, cytotoxic and antimetastatic effects in breast cancer) which were conjugated to VIP, and were demonstrated to have enhanced cytotoxicity in breast cancer cells [85▪].

VIP may be a promoter of breast carcinogenesis. Rat breast cancer tissue in N-methyl nitrosourea treated rats had a higher density of VPAC1 than did normal breast tissue [110]. In C3(1)SV40T antigen mice, which spontaneously develop mammary cancer, VPAC1 immunoreactivity was detected [98]. In receptor binding studies, specific ^{125}I-VIP binding was inhibited by (Lys^{15}, Arg^{16} and Leu^{27}) VIP$^{1-7}$GRF$^{8-27}$ but not RO25–1553 suggesting that VPAC1 binding sites were abundant. Administration of a VPAC antagonist to the mice reduced the mammary tumor burden and altered the proteomic profile in the mammary tumors [98]. Retinoic acid, a chemo-preventive agent, downregulates VPAC1 expression in breast and lung cancer cells [111,112].

VIP/PACAP: PROSTATE CANCER

VIP/PACAP receptors are present in 88–100% of prostate cancers, each of the three subtypes are found in different studies; however, the predominant subtype is VPAC1 [46,113]. In contrast to normal prostate tissue in which for the PAC1 receptor, the SV1/SV2 cassettes and the PAC1 null are the predominant isoforms present, in prostate-cancer the PAC1 null isoform predominates [114,115].

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Addition of VIP to prostrate cancer-cells stimulates cAMP generation; increases the transactivation of HER2/Neu, as well as the transactivation of the androgen receptor via a PKA extracellular signal-regulated kinase mechanism and increases the expression of c-Fos, VEGF and COX-2, the latter dependent on activation of EPAC, NF-kB, PI3K and extracellular signal-regulated kinase (ERK) [116▪,117–121]. VIP has a carcinogenic potential as it induces the malignant transformation of the human prostate nontumor epithelial cell-RWPE-1, with stimulation of matrix metalloprotease (MMP)2, MMP9, cyclin D1; decreased E-cadherin-mediated-cell-cell adhesion, increased cell motility and cell proliferation [122]. Similarly, in prostate cancer cell-lines [123,124], VIP stimulates cell proliferation and invasiveness via a NF-kB mechanism; increases expression of cyclin D1, MMP2 and MMP9; increases expression of the angigenic factor VEGF and COX-2, decreases cell adhesion and E-cadherin; suggesting that VIP may act as a cytokine in early metastatic stages of prostate cancer [123–125].

There are limited data only on the use of VPAC1 overexpression on prostate cancer cell for tumor imaging. In one study [77], PET imaging for localization of prostate cancer cell xenografts (PC3 cell) was reported. In this study [77], ^{64}Cu-TP3939 (a VIP agonist analogue) was synthesized and the prostate cancer xenografts demonstrated enhanced uptake in nude mice and the ratio of the uptake in tumors to normal tissue was four-fold and 2.7-fold increased at 4 and 24 h. Furthermore, in transgenic mice which spontaneously develop prostate cancer, ^{64}Cu-TP3939 imaged histological grade IV prostate intraepitheleal neoplasia, whereas 18Fluoro-deoxy-glucose uptake PET and CT scanning did not [77].

VIP/PACAP: COLON CANCER

VIP/PACAP-receptors are reported in 96% of colon adenocarcinomas [46] by autoradiography. In one study, 35% of well differentiated colon cancers had VPAC1, whereas 65% of moderately differentiated tumors and 87% of poorly differentiated tumors [126▪]. Furthermore, in cancer cells with the highest overexpression of VPAC1, there was increased translocation of activated EGFR to the cell's cytoplasm [126▪]. In addition, overexpression of VPAC1 in colon cancer specimens is found in blood vessels surrounding the tumor and in associated macrophages [126▪].

In a number of different colonic cancer cell-lines, high-affinity binding with VIP is seen [127,128]. Activation of VPAC/PAC1 receptors on various colonic cancer-cells increases cyclic AMP, alters cellular calcium, activates chloride activated Ca2+ channels (HT29 cell) [129], stimulates ornithine decarboxylase activity [130] and stimulates growth [130,131]. In other colonic cancer cells, VPAC-receptor activation inhibits invasiveness and growth [128,132]. Inhibition of VPAC1-receptors inhibits colonic growth of some colonic cancer cell-lines [133].

Numerous studies demonstrate an important connection between inflammation and tumorigenesis, particularly in the case of colonic cancer [134]. The importance of VIP/PACAPs anti-inflammatory activities on the colonic tumorigenic process has been shown in recent studies [135,136]. In PACAP-deficient mice (PACAP-KO mice) [135] treated with dextran sulfate sodium, an established model of colitis, the PACAP-KO mice developed more severe inflammation [135,137], and 60% developed aggressive colorectal cancers, whereas none of the controls treated similarly developed colon cancers. APC mice, a model of spontaneous colon cancer, exhibit increased colonic inflammation and a defect in expression of both VIP and PACAP, which has led to the suggestion that this could be responsible for the increased proinflammatory environment in these animals and the increased initiation and progression of colonic cancers they develop [136].

The overexpression of VPAC-receptors by colon cancer has been investigated both to image the tumors and for targeted therapy by coupling cytotoxic agents to VIP.

In one study [83], colon cancer patients, tumors were imaged using ^{123}I-VIP in 87 and 82% of patients with primary and relapsing cancers, respectively [83]. VIP scans were positive for 100% of the lymph node metastases and 89% of patients with liver metastases. However, in another study, no imaging of colonic cancers could be seen, and this was attributed to their low expression of VPAC-receptors [80]. ^{18}F-(R^{8,15,21}, L^{17})VIP was used to localize C26 colon cancer tumors in nude mice [74]. In nude mice-bearing HT29 tumors, a 15-mer phosphorothioate antisense construct to the c-myc oncogene mRNA, which was conjugated to VIP-polylysine, strongly reduced colonic cancer xenograft proliferation [89].

VIP/PACAP: CENTRAL NERVOUS SYSTEM TUMORS

In various human gliomas (astrocytomas, ependymomas and oligodendrogliomas), PAC1 receptors are present in 81–100% [138,139] and in 20% of meningiomas [139]. PAC1 binding sites, but not VPAC sites, were found on glioma cell membranes [138]; PAC1 receptor mRNA was significantly

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overexpressed in glial tumors, especially in oligodendrogliomas [57] and PACAP stimulated activation of adenylate in glial cell membranes [138,139]. In various glioma cell lines, VPAC1 and VPAC2 receptors are also found [140] with the VPAC1 receptor localized strongly to the nucleus, and there was a positive correlation between the degree of VPAC1 nuclear localization and glioma grade [140]. In two glioblastoma multiforme cell lines [MO59K and MO59J] [141], activation of VPAC and PAC1-receptors inhibited cell migration, but had no effect on cell proliferation. Furthermore, increasing expression of VPAC receptors resulted in decreasing levels of cell invasion in these glioblastoma cell lines and this inhibitory action was found to be mediated by inhibition of AKT signaling [142▪]. Furthermore, in three other glioblastoma multiforme cell lines (U87, U118 and U373), PAC1, but not VPAC-receptors were found [143], and PACAP activated adenylate cyclase in these cells, as well as increased cytosolic Ca^{2+}, and a PAC1 antagonist inhibited proliferation of each of the three glioblastoma multiforme cell lines [143].

In childhood, primitive neuroectodermal tumors (medulloblastomas, etc.) are one of the most frequent groups of CNS malignant tumors [144]. In one study [144], 86% of the central primitive neuroectodermal tumors had VPAC1 mRNA and 75% had VPAC2 mRNA. Furthermore, the addition of VIP to 10 primitive neuroectodermal tumor cell lines inhibited growth in 70% [144]. These tumors also possess PAC1 receptors [64,145]. In studies of mutant mice exhibiting an enhanced development of medulloblastomas, generally hedgehog signaling and PKA play opposing roles [145,146▪]. Deletion of single copy of the PACAP gene in these mutant mice (ptc1 mice) results in 2.5-fold increase in the development of medulloblastomas, demonstrating that PACAP exerts a powerful inhibitory effect, which is PKA-dependent, on the induction, growth or survival of this tumor [145]. Primary tumor spheres made from these medulloblastomas exhibited constitutive hedgehog signaling [64,145]. The addition of PACAP to these medulloblastoma primary tumorspheres antagonized the hedgehog signal, which was blocked by a PKA inhibitor, and PACAP-inhibited proliferation, suggesting that the regulation of hedgehog signaling in these tumors by PACAP administration might prove a novel therapy [64].

Whereas a number of other studies have also reported the activation of PAC1 or VPAC1 receptors on various glial cell lines inhibits their proliferation, still other studies report that the activation of these receptors on these tumor cells increases growth [54▪,65,147,148].

VIP/PACAP: NEUROBLASTOMAS

Neuroblastoma, an embryonal tumor arising from the sympathetic nervous system, is the most frequent neonatal malignancy and is also common in early childhood. Almost all neuroblastoma-tumor cells possess at least one of the VPAC/PAC-receptors, with VPAC1 receptors reported in 60–100%, VPAC2 in 31% and PAC1 in 60–100% [149–151]. In addition, PACAP-mRNA is frequently detected in neuroblastoma cells (60–100%) [149,151], whereas VIP-mRNA is less frequently detected (12–75%) [149,151]. In a study of six neuroblastoma tumor cell lines, PAC1 receptors were detected in the highest amounts and were present in 100% of the cell lines, whereas VPAC2/VPAC1 were present in low amounts in four of six (66%) and two of six (33%) of the neuroblastoma cell lines [152]. Detailed studies of one neuroblastoma cell line (SH-SY5Y cells) demonstrated that multiple different PAC1 receptor splice-variants are present involving the third intracellular receptor domain (ic3) as well as the aminotermial receptor domains (N-terminal) [152]. The null-ic3 splice variant was the predominant form found [152]. Different PAC1 splice variants can respond with different potencies, affinities, efficacies to VIP/PACAP [7], and this was seen with different SH-SY5Y-splice-variants [152]. This variation in the expression of different PAC1 splice variants is reported to be a mechanism of fine tuning of brain activity [153▪]; however, what role it plays in different neuroblastoma tumor cells is unclear.

In neuroblastoma cells as well as neuroblastoma cell lines, PACAP activates phospholipase-C [152], and stimulates VIP gene transcription [154,155] and c-Fos expression [155]. PAC1 stimulation of c-Fos/VIP expression is dependent on PAC1-activation of PKA, PKC/ERK1/2 [155]. Furthermore, PAC1 stimulation of c-Fos requires calmodulin [156]. Both PACAP/VIP can activate adenylate-cyclase in various neuroblastoma tumor cells [150,152,154].

VIP/PACAP can have a marked effect on the growth/differentiation of various neuroblastoma tumor cells. VIP/PACAP stimulate growth/proliferation of neuroblastoma tumor cells [157,158] and induce differentiation and neuritogenesis [158,159]. The neurotrophic effects of PACAP on the neuroblastoma tumor cell, SY5Y are mediated by cAMP-dependent processes involving Epac-dependent activation of ERK and activation of the p38 kinase [50]. Furthermore, 22% of neuroblastoma tumors overexpress MYCN and this is associated with a poor-prognosis [160]. VIP treatment of neuroblastoma tumor cells *in vitro* [160] decreases by 25% MYCN expression and it has synergistic effects with retinoic acid, which is used in the therapy of

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neuroblastomas [160]. This result suggests that VIP in combination with retinoic acid may have potential benefit in patients with neuroblastomas overexpressing MYCN [160].

VIP/PACAP: PANCREATIC CANCER

Pancreatic ductal cancer frequently expresses VIP/PACAP receptors (65%) [46].

VPAC1 mRNA is present in human pancreatic-cancer cell-lines such as CAPAN-2 [161]. VIP increases cAMP/c-fos mRNA in CAPAN-2 cells which is antagonized by VPAC1 inhibitors [161]. Furthermore, proliferation of CAPAN-2-cells and tumors in nude mice is inhibited by VPAC1 inhibitors [161].

VIP hypersecretion by neural tumors such as ganglioblastomas/neuroblastomas in children or non-β-cell pancreatic tumors (VIPomas) causes a characteristic clinical syndrome (Verner–Morrison syndrome) [162]. VIPomas cause large-volume, watery-diarrhea, hypokalemic and achlorhydric [162]. A recent study demonstrates that VIPoma-cells express both VPAC1 and VIP-IR, raising the possibility that the hypersecreted VIP could be having an autoregulator effect on the tumor cells [163].

Neuroblastoma tumors occasionally hypersecrete VIP causing watery diarrhea and the VIPoma syndrome [164]. These occur in children, approximately 80 cases have been described, and it usually occurs in well differentiated neuroblastomas [164].

VIP/PACAP: OTHER TUMORS

A number of other tumors have also been described in which PACAP/VIP effect (stimulatory/inhibitory) their growth, but these are, in general, less well studied than the tumors reviewed above and will not be covered in this review. These include pheochromocytomas [63,165], gastric cancer [166,167▪], cervical cancer [168], choriocarcinoma cells [169], neuroendocrine tumors [60▪,62,170] and renal cancer [59,171,172▪].

CONCLUSION

A large number of different neoplasms possess and overexpress receptors for VIP/PACAP. In some tumors, activation of these receptors has growth stimulatory effects and in others they have growth inhibitory effects. Furthermore, in a number of different tumors, VIP/PACAP have an autocrine function with the tumor both possessing VIP/PACAP receptors and secreting the active peptide.

Recent studies, reviewed here, are providing increased insights into the cellular signaling cascades mediating the growth effects of this family of receptors on both normal tissues and tumors. Particularly important in a number of cases is their ability to transactivate the EGF family of receptors.

The overexpression of VPAC/PAC receptors is receiving increased attention with many of the tumors, not only as a means to imaging their location and extent, but also as a method to target cytotoxic agents to these tumors. This receptor family is receiving an increased attention because of the success of using this approach with radiolabeled somatostatin analogues for a wide range of neuroendocrine tumors and because the VIP/PAC family of receptors are overexpressed on a number of common tumors that do not overexpress somatostatin receptors.

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Conflicts of interest

There are no conflicts of interest.

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